## REMARKS

Upon entry of the amendments, claims 31-32 and 34-43 are pending. Claims 30 and 33 are cancelled. Applicants reserve the right to pursue the subject matter of these claims in one or more continuing applications. Claims 31-32 and 34-35 are amended to correct dependency. New claims 36-43 are added. Support for these new claims can be found in cancelled claims 30 and 33 and in the specification at page 22, lines 8-19; page 22, line 20 - page 23, line 5; page 23, lines 6-13. No new matter is added.

In support of the remarks and arguments stated *infra*, Applicants have submitted herewith the Declaration of Dr. Jonathan S. Stamler under 37 C.F.R. §1.132 ("Stamler Declaration").

**Rejection under 35 U.S.C. §112, First Paragraph**

Claims 4-6 and 30-35 are rejected under 35 U.S.C. §112, first paragraph for failing to comply with the enablement requirement. The Examiner states that Applicants have not fully enabled the claimed invention for the full scope of the claim. Specifically, the Examiner states that the claims are drawn to "conditions" that allow the formation of S-nitrosohemoglobin (SNOHb). However, the Examiner asserts that specific conditions are necessary to make the desired end product and the instant specification does not describe all the desired conditions to achieve SNOHb. Moreover, the Examiner asserts that the results of the instant application describe that the desired SNOHb is not obtained under the described experimental conditions (*e.g.*, Example 3). As such, the Examiner contends that the one of ordinary skill in the art would be burdened with undue experimentation to determine the appropriate conditions for obtaining SNOHb rather than obtaining methemoglobin (metHb) or iron-nitrosylhemoglobin. *See*, Final Office Action at pages 2-3 and Advisory Action at page 2. Applicants traverse with respect to the claims as added and amended herein.

The present invention is directed to the novel discovery that nitric oxide (NO) binding to oxygenated hemoglobin (oxyHb) is cooperative *in vivo*, which is in direct contradiction to previous studies which suggested such interaction is non-cooperative. *See*, specification at page 15, line 22 - page 16, line 12. The present invention describes that the distribution of the hemoglobin population having vacancies on the hemes

controls the function of hemoglobin. Thus, the present invention provides that by regulating the functional behavior of this vacancy population, hemoglobin can either a) quench and eliminate excess nitric oxide (*i.e.*, form metHb), b) store excess nitric oxide in a form that is not a donor of NO (*i.e.*, iron nitrosylhemoglobin), or c) store NO in a form that donates NO (*i.e.*, SNOHb).

More specifically, the instant specification describes two conditions essential to modulate the interaction between NO and oxyhemoglobin. First, for storage of NO (either in non-donor form as iron nitrosylhemoglobin or in donor form as SNOHb), the R structure of hemoglobin must be maintained. The instant specification further describes that the R structure of hemoglobin can be maintained under low phosphate conditions (*i.e.*, less than 100 mM and preferably 10mM). Second, for the generation of NO in donor-form as SNOHb, the redox chemistry of hemoglobin must be preserved to permit the transfer of NO from the heme Fe to cysteine on the β subunit. These two conditions are illustrated in Examples 3 and 4. *See*, Stamler Declaration at ¶ 5.

The experiments described in Example 3 were designed to measure the yield of iron-nitrosylhemoglobin vs. metHb under low phosphate conditions (*i.e.*, less than 100 mM and preferably 10mM), plus or minus borate. Borate is a unique compound which inhibits cysteine NO-reactivity and prevents the transfer of NO from heme. Thus, the addition of borate to the low phosphate conditions is used to assess the second step described above; that is, the ability to form SNOHb following the transfer of NO from the heme Fe to cysteine on the β subunit.

The experiments in Example 3 were not designed to measure the yield of SNOHb under those conditions. *See*, page 46, line 13 - page 47, line 11. The results in Example 3 demonstrate that under low phosphate conditions (plus or minus borate) formation of iron-nitrosylhemoglobin is favored over metHb. These results demonstrate the first essential condition: that maintenance of the R structure of hemoglobin favors the storage of NO (either in non-donor form as iron nitrosylhemoglobin or in donor form as SNOHb). However, these measurements do not measure the difference between iron nitrosylhemoglobin or SNOHb under the low phosphate conditions (plus or minus borate). Had metHb, iron-nitrosylhemoglobin and SNOHb been analyzed simultaneous under the conditions of Example 3, metHb formation would have been favored under

high phosphate conditions, iron-nitrosylhemoglobin formation would have been favored under low phosphate conditions plus borate and SNOHb formation would have been favored under low phosphate conditions in the absence of borate. *See*, Stamler Declaration at ¶ 6.

The experiments described in Example 4 were designed to measure the yield of SNOHb under low phosphate conditions in the absence of borate and the results of those experiments show that SNOHb and intraerythrocytic SNOHb are produced under those low phosphate conditions in the absence of borate. *See*, page 18, lines 16-25; page 47, line 12 - page 48, line 2; Figures 4C and 4D and page 8, lines 19-22. The results in Example 4 demonstrate that for the generation of NO in donor-form as SNOHb, the redox chemistry of hemoglobin must be preserved allowing the cysteine to display NO-reactivity to permit the transfer of NO from the heme Fe to cysteine on the β subunit. *See*, Stamler Declaration at ¶ 7.

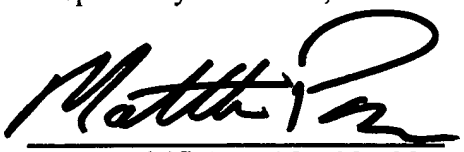
The pending claims as added and amended herein recite the necessary conditions for the formation of SNOHb or intraerythrocytic SNOHb: (1) maintaining the R structure of hemoglobin and (2) preserving the redox chemistry or regulating the auto-oxidation of hemoglobin. The pending claims also specify that these conditions comprise low phosphate and the absence of borate, respectively.

For the foregoing reasons, Applicants submit that one of ordinary skill in the art, provided with the teaching of the instant specification, would readily determine the appropriate conditions for producing SNOHb and intraerythrocytic SNOHb without the need for undue experimentation. Applicants respectfully request the rejection be withdrawn.

## CONCLUSION

On the basis of the foregoing amendment and remark, Applicants respectfully submit that the pending claims are in condition for allowance. Should any questions or issues arise concerning this application, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,



Ivor R. Elrifi, Reg. No. 39,529
Matthew Pavao, Reg. No. 50,572
Attorneys for Applicants
c/o MINTZ LEVIN
Tel.: (617) 542-6000
Fax: (617) 542-2241
**Customer No.: 30623**

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